

WU-NK-101: an Enhanced NK Cell Therapy Optimized for Function in the Tumor Microenvironment (TME)

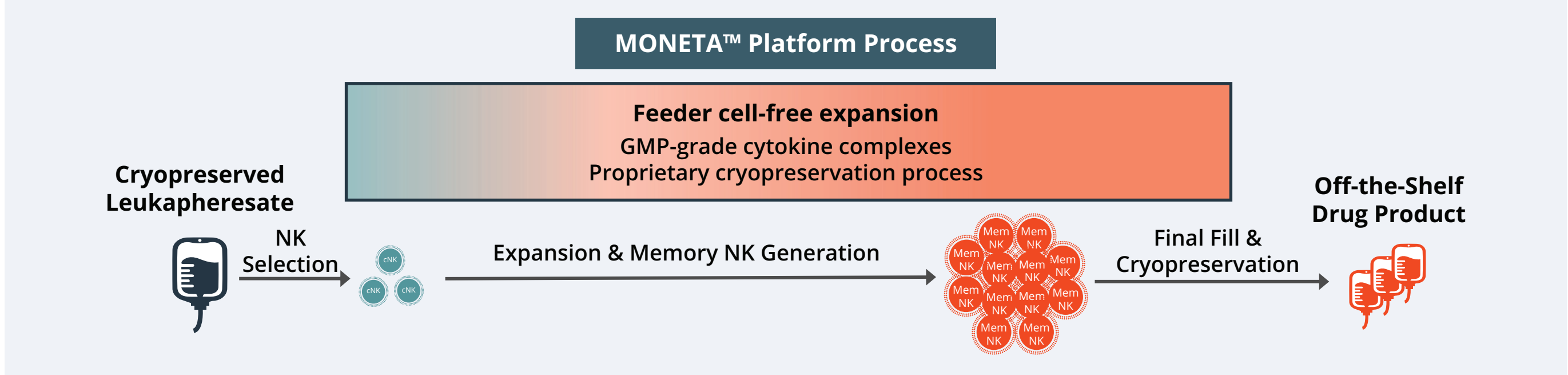
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Background

- Natural Killer (NK) cells, identified as CD3^{neg}/CD56^{pos} or CD3^{neg}/CD56^{dim}/CD16^{pos} lymphocytes, exist in peripheral blood, bone marrow, spleen, lymph nodes
- NK cells are functionally defined as cytotoxic and/or cytokine secreting in response to tumor cells
- NK cells can identify and eliminate virus-infected cells and tumor cells without prior sensitization
- The efficacy of adoptive cell therapies (ACTs) against solid tumors has been limited by identification of target antigens, restricted trafficking to tumors, and establishment of a highly immuno-suppressive tumor microenvironment (TME), aiding in tumor escape and progression
- Efforts are being focused on enhancing NK metabolic fitness and anti-tumor function within a nutrient-restrictive TME
- WU-NK-101, manufactured using the MONETA™ platform, is an ACT product derived from healthy-donor (CD3^{neg}/CD56^{pos}) NK cells to provide enhanced cytotoxicity and metabolic adaptability, addressing current challenges of ACT in the setting of an adverse TME

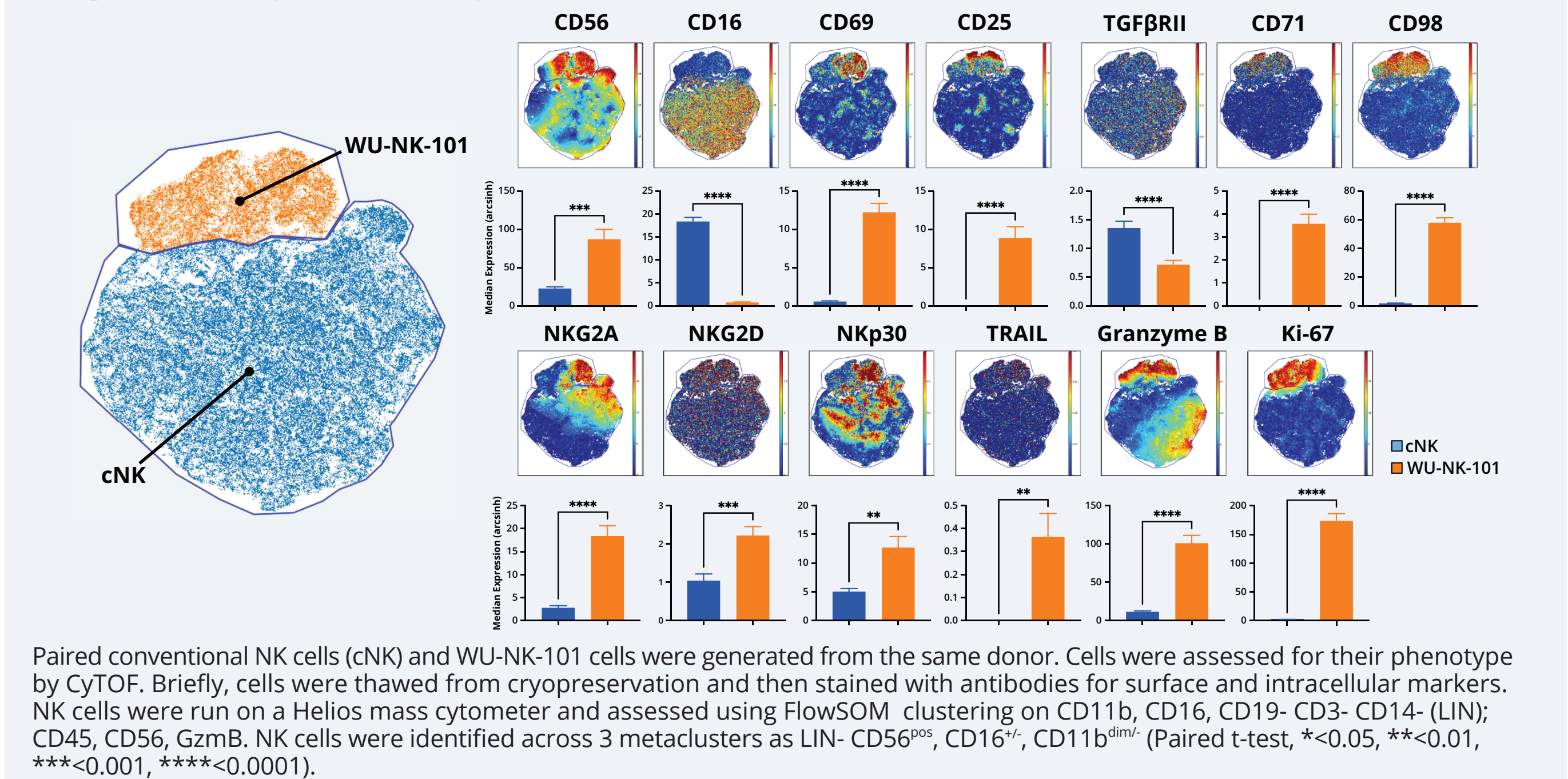


Methods

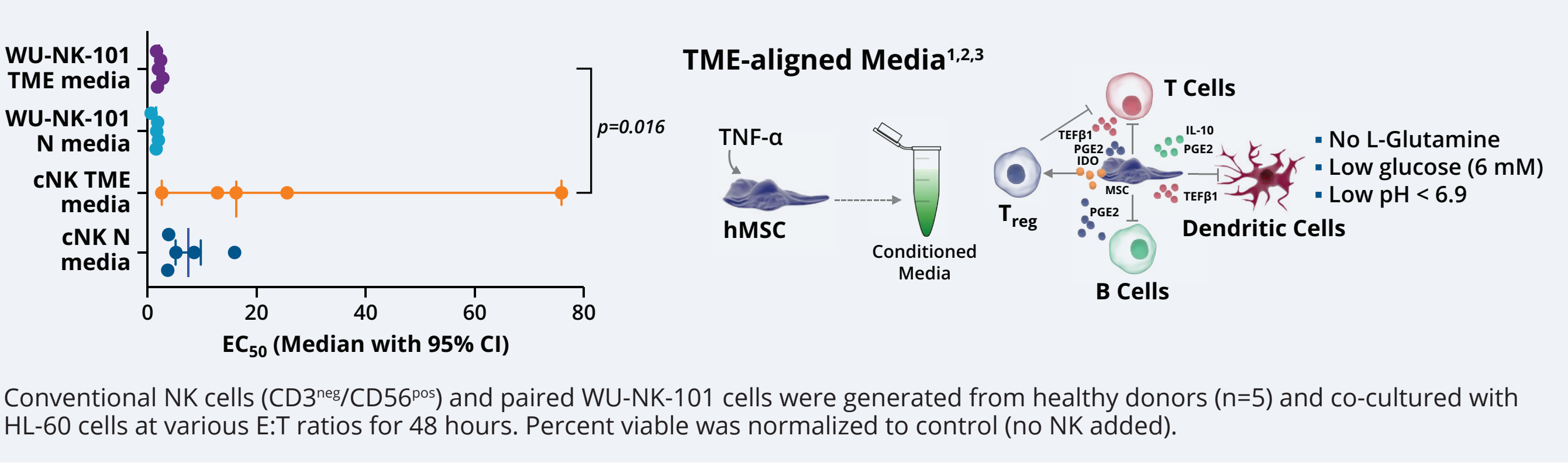
- WU-NK-101 cells were phenotypically characterized using multidimensional mass cytometry (CyTOF)
- Cytotoxicity was assessed in vitro in 2 culture conditions, complete media (N media; IMDM medium [glucose 25 mM, neutral pH 7.2–7.4]) supplemented with 20% fetal bovine serum (FBS) and antibiotics, and TME-aligned media. TME-aligned media is composed of custom RPMI (pH 6.9, glucose 6 mM) supplemented with human serum, human platelets lysate, antibiotics and cytokines from TNF α stimulated human mesenchymal stem cells (hMSC) culture. Stimulated MSCs secrete soluble molecules, such as nitric oxide, PGE2, IDO, IL-10 and TGF β 1.^{1,2,3}
- Multi-omic studies were performed by mass spectrometry-based proteomics
- In vitro intrinsic and antibody-dependent cellular cytotoxicity (ADCC) killing assays (IncuCyte, Sartorius): WU-NK-101 cells were co-cultured with tumor cells and vehicle, isotype control antibodies, or trastuzumab over 72 hours
- In vivo: SKOV3-CBR-GFP cells were inoculated (IP) in NSG mice. Mice were then injected with WU-NK-101 cells + trastuzumab or isotype control
- Cell trafficking/penetration to TME was measured in NSG mice (SKOV-3) by tracking fluorescently-labeled WU-NK-101 cells \pm trastuzumab to enhance killing of trastuzumab-targeted cells
- Metabolic fitness was assessed by Seahorse Real-Time Cell Metabolic Analysis (Agilent)

Results

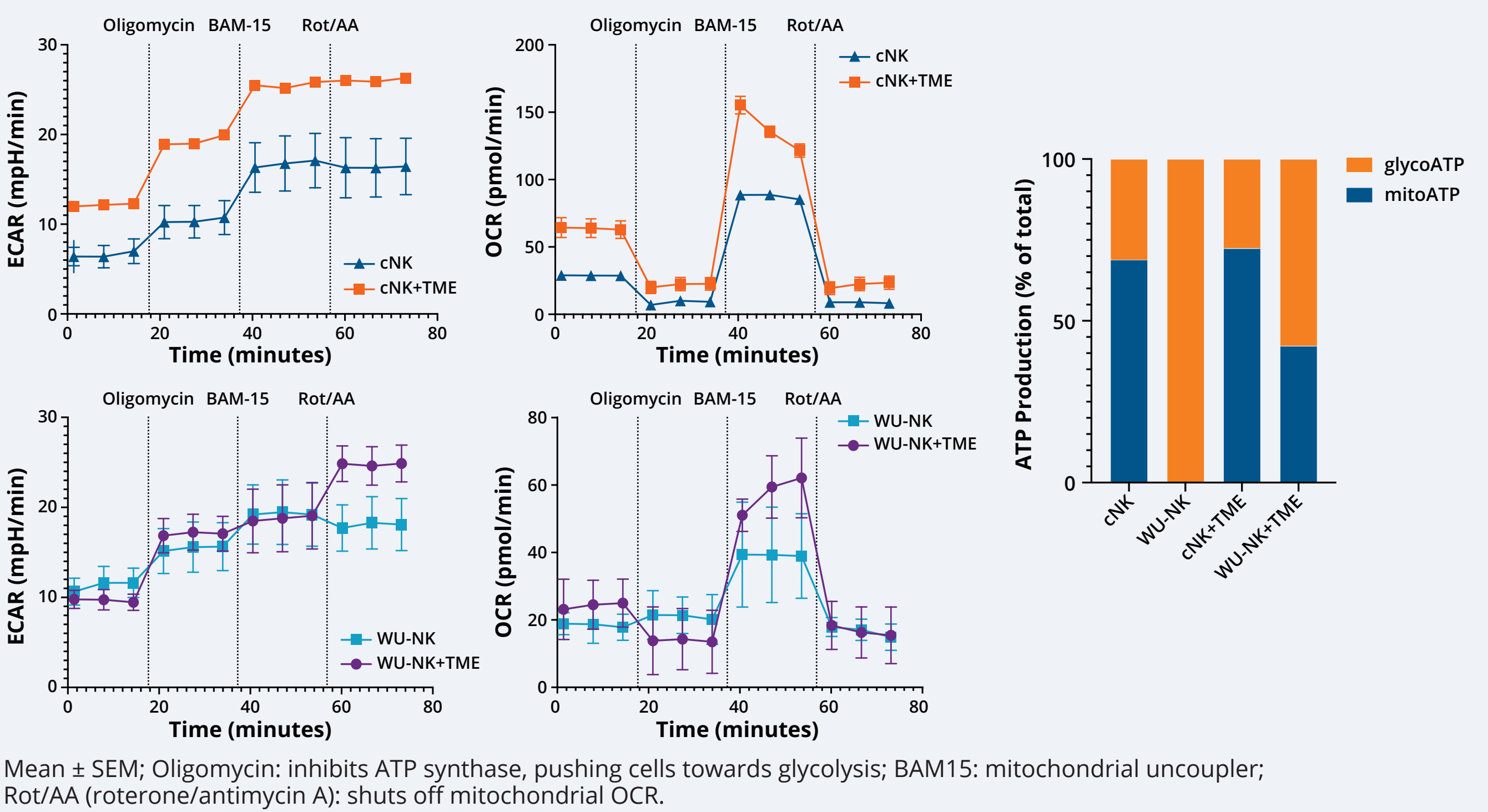
WU-NK-101 Has a Unique Phenotype Optimized for Rapid Activation and Improved Cytotoxicity



WU-NK-101 Cytotoxicity is Not Hampered by Adverse TME as Compared to cNK Cells

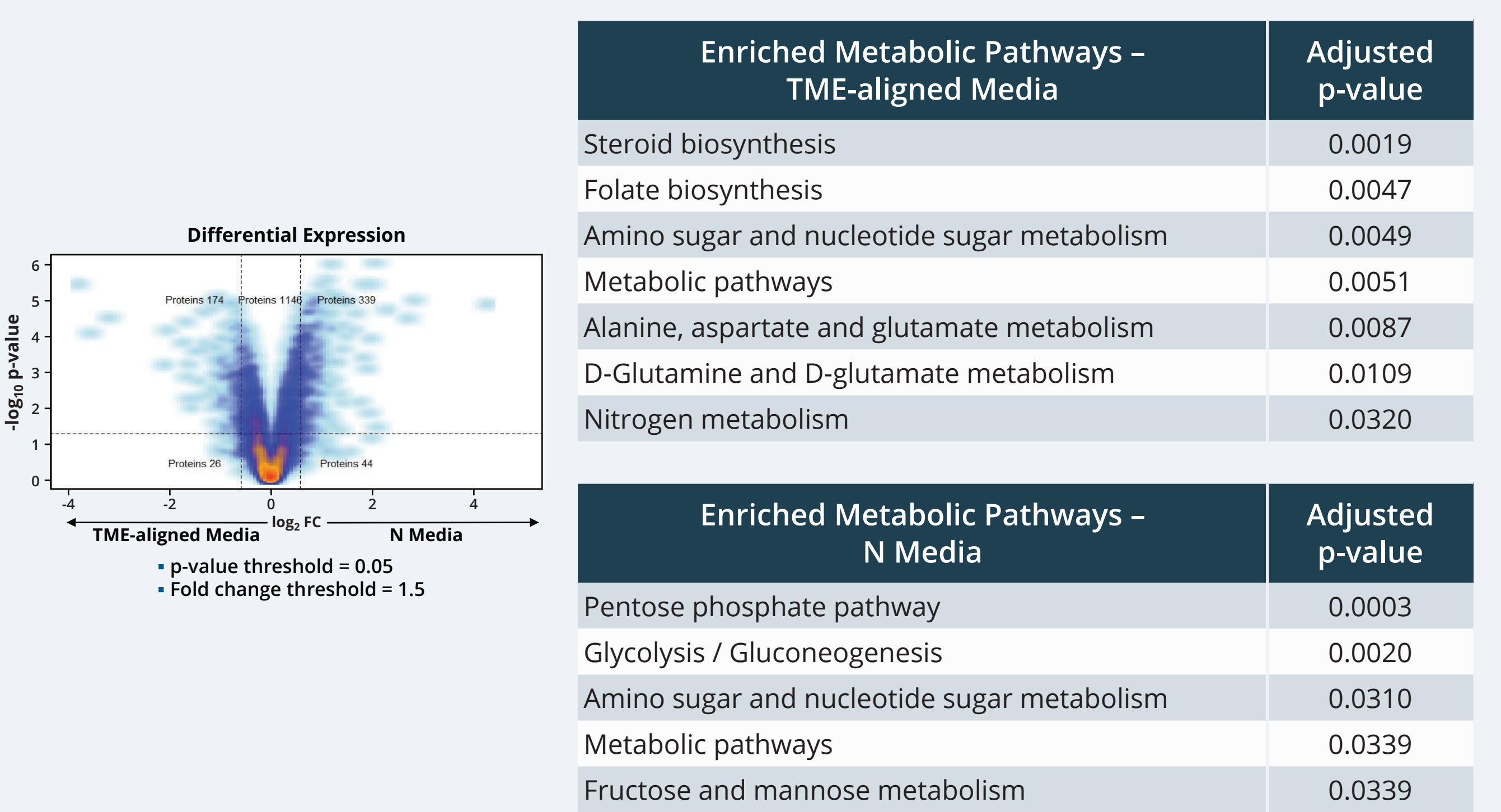


Compared to cNK, WU-NK-101 Has Improved Metabolic Fitness in TME



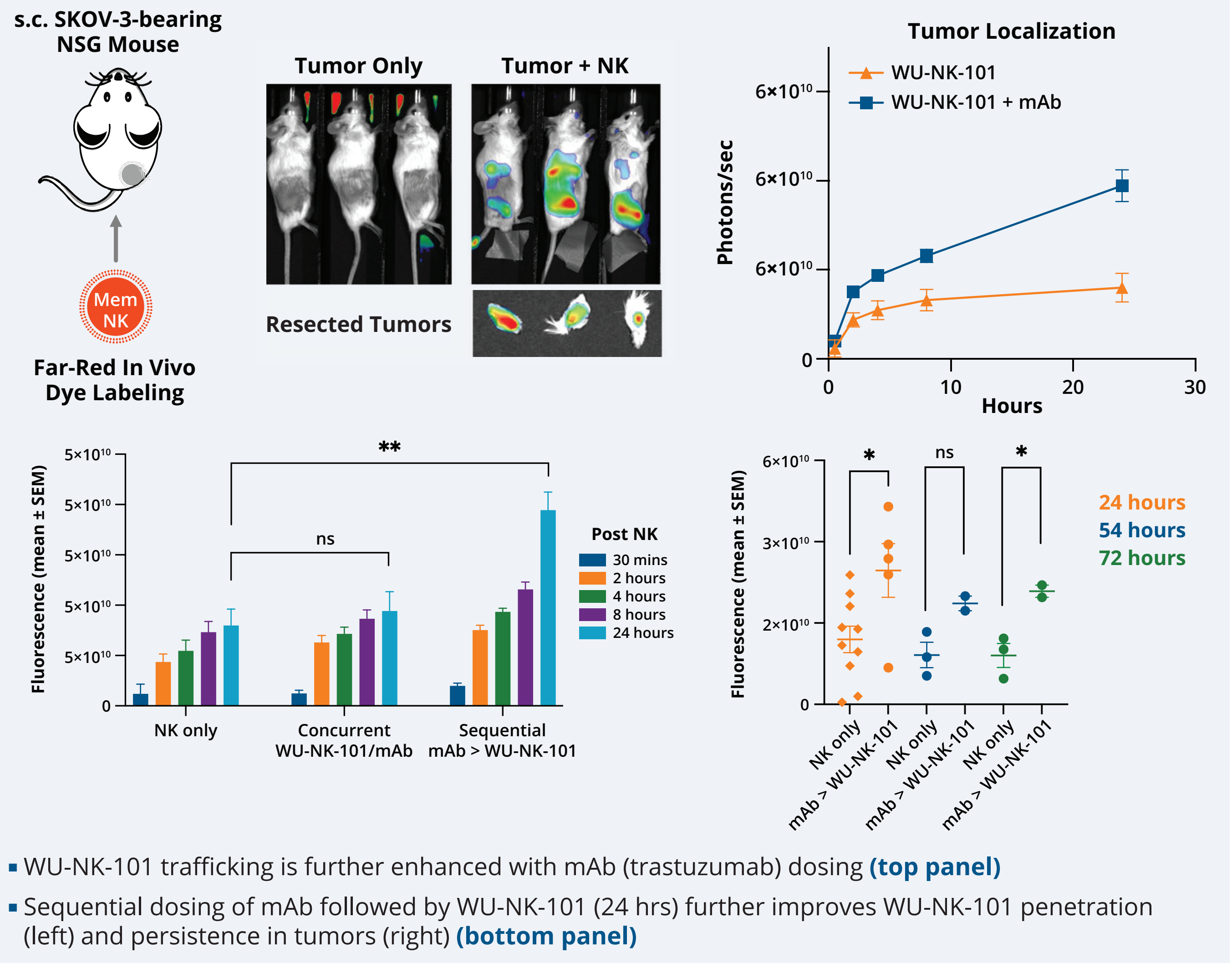
- WU-NK-101 exhibited enhanced glycolytic and mitochondrial oxidative phosphorylation capacity (i.e., metabolic fitness) compared to cNK cells
- WU-NK-101 had metabolic profile consistent with aerobic glycolysis (“Warburg metabolism”), which may abrogate the adverse effects of an inhibitory TME
- Compared to cNK, WU-NK-101 extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were not greatly impacted by the TME

KEGG Pathway Analysis Suggests Metabolic Flexibility of WU-NK-101



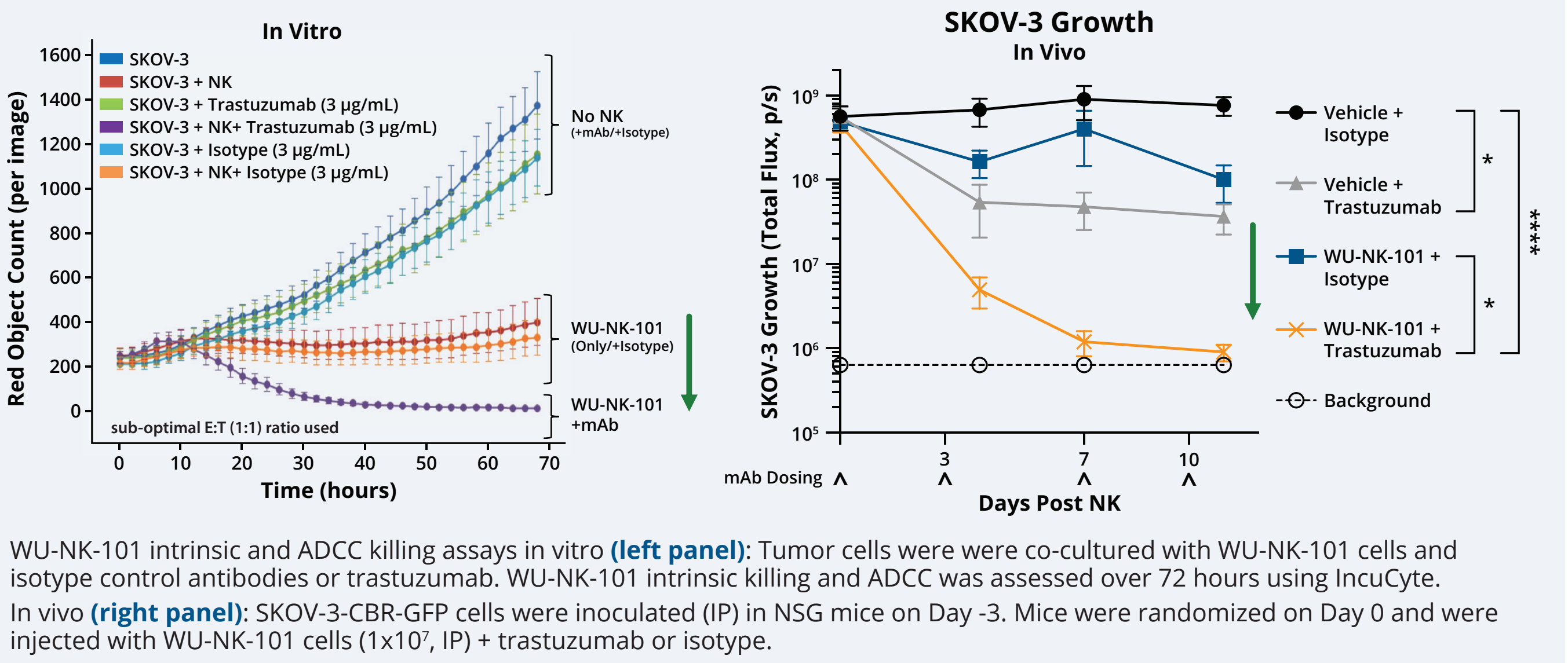
- Metabolic flexibility aids in function within the TME. In N media, WU-NK-101 used glucose as its main nutritional source; in hypoglycemic TME-aligned media, amino acid metabolic pathways were upregulated, which augurs metabolic adaptability

WU-NK-101 in Combination with mAb Increases Intra-Tumor Infiltration and Persistence



- WU-NK-101 trafficking is further enhanced with mAb (trastuzumab) dosing (top panel)
- Sequential dosing of mAb followed by WU-NK-101 (24 hrs) further improves WU-NK-101 penetration (left) and persistence in tumors (right) (bottom panel)

mAb Combination Enhances Anti-Tumor Function of WU-NK-101 by ADCC



Conclusions

- WU-NK-101 exhibited enhanced/adaptive metabolic fitness contributing to resilience to an adverse, highly-immunosuppressive TME, relative to cNK cells
- WU-NK-101 showed potent cytotoxicity against tumor cells in vitro and in vivo, which could be further enhanced by ADCC
- WU-NK-101 in combination with mAb enhanced trafficking and tumor penetration, contributing to anti-tumor activity
- These data suggest that WU-NK-101 may overcome current limitations of adoptive cellular therapies and support clinical evaluation of NK cell-based approaches in the setting of solid tumors

References
1. Samsonraj et al. *Stem Cells Transl Med.* 2017 Dec;6(12):2173-2185. 2. Sasser et al. *Cancer Lett.* 2007 Sep 8;254(2):255-64. 3. Studebaker et al. *Cancer Res.* 2008 Nov 1;68(21):9087-95.
Conflict of Interest
Sergio Rutella's lab at Nottingham Trent University received research support from Wugen, Inc.
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